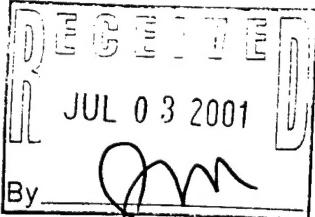


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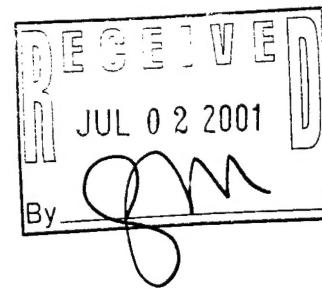
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Research Agreement No. DAAD19-99-1-0037
Instrumentation for Chemical Studies of Biomimetics



Technical Report:

The research work for this proposal has proceeded extremely well and significant progress has been made. As outlined in the original proposal, the initiative features collaborative interactions among several core faculty in bioorganic and biophysical chemistry to share expertise, technology, and ideas. We are happy to report that collaborations among, not only these faculty, but also numerous other groups on campus has resulted from the acquisition of the equipment. Together, these faculty have brought fundamental studies of protein-nucleic acid and protein-protein interactions as well as interactions of proteins with prosthetic groups and ligands to bear on key structure-function questions with potential applications to biocatalysts, biomaterials, and biosensors. The orginal budget request listed five instruments to support chemical studies of biomimetics. The revised budget was pared down to three major instruments. After extensive evaluation and discussion among the co-P.I.s and other users, we concluded that the combined specifications of high resolution circular dichroism (CD) spectra and high signal-to-noise stopped-flow kinetic data were not available in a single instrument. We therefore purchased four instruments as described below. We also purchased an ultracentrifuge rotor for protein purification.

CD Spectrometer: Jasco J-800 spectropolarimeter equipped with a model PFD-335S Peltier temperature control system, model JWATS-489 automatic titration unit, 6 short path rectangular cells, and other accessories (computer, etc.). The choice of instrument was largely based on the excellent performance of the Jasco on our samples coupled with a trade-in that lowered the price. The instrument was installed in November 1999 and has been used round the clock by numerous investigators on campus. It is housed in Dr. Zagorski's (Co-P.I.) newly renovated laboratory.

The Zagorski group has been using the CD to study binding of small molecule inhibitors of amyloid formation, which is a more rapid method than NMR for exploring possible conformational changes of the A β peptide. Compounds resembling detergents may inhibit β -aggregation by encouraging a conformational conversion favoring monomeric A β . Detergents such as SDS that have >12 carbons and a negative or positive charge inhibit β -amyloid fibril formation, which according to their work is the result of a β -sheet \rightarrow α -helix or random extended chain \rightarrow α -helix conversion. Their initial studies re-examined the effects of free fatty acids hexanoic acid (6 carbons) and *cis*-oleic acid (18 carbons). Hexanoic acid had no effect on the structure of A β , while oleic acid induced a random extended chain \rightarrow β -sheet conversion. Interestingly, although the presence of β -sheet structure is consistent with amyloid-like material, they did not detect any β -amyloid fibrils. These studies will be continued with other fatty acids. Manuscripts reporting the results completed so far are listed below.

"Melatonin Reverses the Pro-Fibrillogenic Effects of Apolipoprotein E4 with the Alzheimer Amyloid A β Protein," B. Poeggeler, L. Miravelle, M. G. Zagorski, T. Wisniewski, Y.-J. Chyan, Y. Zhang, H. Shao, T. Thomas, B. Frangione, J. Ghiso, and M. Pappolla, *J. Biol. Chem.* in press.

"Amyloid A β (1-40) and A β (1-42) Adopt Remarkably Stable and Random Extended Structures in Water Solution at Physiological pH," H. Shao, Y. Zhang, L. Hou, N. Menon, E. E. Neuhaus, J. Brewer, M. P. Vitek, R. A. Makula, A. Przybyla, and M. G. Zagorski, *J. Am Chem. Soc.*, submitted.

Stopped-flow Spectrophotometer: Kin-Tek SF-2001 stopped-flow spectrophotometer equipped with xenon arc lamp, two fluorescence detectors, anisotropy measurement capability, photomultiplier, and CCD array detector. This instrument allows simultaneous measurement of

fluorescence and absorbance. In addition, the CCD array detector renders us the capability to collect spectral data as a function of time. The instrument was installed in June 2000. It is housed in Dr. Lee's (Co-P.I.) newly renovated laboratory.

The Lee research group routinely performs transient kinetic analyses of an ATP-mediated peptidase reaction by *Escherichia coli* Lon protease using the stopped-flow apparatus. These studies have shown that the rate-limiting step of peptide hydrolysis is either initial binding of substrate or a conformational change of the enzyme-substrate complex prior to cleavage. Further stopped-flow experiments are currently underway to determine the rate constants of substrate binding and peptide hydrolysis to further define the rate-limiting step of the reaction. The data collected by the stopped-flow apparatus will be crucial to establishing the minimal kinetic mechanism of the ATP-dependent peptide cleavage reaction by Lon.

The Barkley group is using the stopped-flow apparatus to study the mechanism of DNA synthesis by HIV reverse transcriptase. Quenched-flow experiments suggested that a non-productive complex forms between the enzyme and substrate and that a conformational change in the enzyme is the rate-limiting step. Fluorescence stopped-flow experiments are underway to measure the rates of release of substrate by enzyme in the absence and presence of catalysis.

Nanosecond Transient Absorption/Raman Spectrometer: Chromex model 250is/sm spectrograph with both CCD and photomultiplier outputs, Andor System 3011 intensified CCD, LeCroy 200 MHz oscilloscope, Quantum Northwest Flash 100 cuvette holder, and other accessories (optical components and mounts, test equipment, computer, etc.). The instrumentation was assembled from components in Dr. Barkley's (P.I.) laboratory and attached to an existing Spectra Physics MOPO pulsed laser system. The complete instrument has been fully tested with the photomultiplier as detector. The ICCD will be installed and tested this summer.

The Barkley group is using the nanosecond absorption spectrometer to measure triplet yields of indole derivatives in nonaqueous solvents as part of their ongoing effort to develop tryptophan as a probe of peptide structure. They are in the process of testing procedures for scrupulous removal of dissolved oxygen, a more serious problem than in their previous studies in aqueous solution. In July 2001, Dr. Clemens Burda, a laser spectroscopist, will join the chemistry department as an assistant professor. He will be using the absorption and Raman instrumentation for his studies of quantum dots. Nanosecond Raman experiments are described below.

Tunable CW Raman Spectrometer: Chromex model 500is spectrograph with Andor CCD, Coherent model 899 ring dye laser, laser table, and other accessories (optical components and mounts, power supply, calibration lamp, computer, etc.). The instrument was assembled from components in Dr. Simpson's (Co-P.I.) laboratory and attached to an existing Spectra Physics Ar+ ion laser. It has been fully integrated into the laboratory and already made a significant impact upon the projects described in the proposal. The new CCD camera has made it possible to more rapidly and efficiently perform both CW and nanosecond Raman experiments.

The Simpson group is using the CW Raman system to obtain high-resolution wavelength dependent measurements of scattering intensity (resonance excitation profiles) from Fe^{II}octaethyl porphyrin-2 methyl imidazole (FeOEP-2MeIm), a model compound for deoxyhemoglobin, and other metalloporphyrin systems. This information can be used to map excited-state distortions and to provide crucial quantification of the vibrational energy distribution information obtained in nanosecond experiments. Preliminary results and their comparison to theory have been presented at a national meeting (ACS Meeting, August 2000). More extensive characterization and testing are currently underway.

The Simpson group is using nanosecond Raman experiments to examine the flux-dependence of the Stokes and anti-Stokes Raman scattering intensities of metalloporphyrins, in

particular FeOEP-2MeIm. The results provide exciting new information about vibrational energy flow in large molecules in solution. Findings have been presented in national (Gordon Conference, 2000; ACS Meeting, August 2000) and regional (local ACS meetings, 2000, 2001) forums. Manuscripts reporting these results are currently in progress.

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